PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

JUL 14 2004

Applicant's or agent's file reference P26557PC00/TWI	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/00340	International filing date *(day/month/year)* 14.01.2003	Priority date *(day/month/year)* 14.01.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/86		
Applicant VERENIGING VOOR CHRISTELIJK WETENSCHAPPELIJK ...		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14.08.2003	30.07.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - Gitschiner Str. 103 D-10958 Berlin Tel. +49 30 25901 - 0 Fax: +49 30 25901 - 840	Authorized Officer Panzica, G Telephone No. +49 30 25901-328

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/00340

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-8, 10-55	as originally filed
9	filed with telefax on 18.06.2004

Claims, Numbers

1-23	filed with telefax on 22.04.2004

Drawings, Figures

1-12	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3, 5-9, 12,13
	No:	Claims	1, 2, 4, 10, 11, 14-23
Inventive step (IS)	Yes:	Claims	5-9, 12, 13
	No:	Claims	1-4, 10, 11, 14-23
Industrial applicability (IA)	Yes:	Claims	1-23
	No:	Claims	-

2. Citations and explanations

   see separate sheet

V. Reasoned Statement

The following documents are referred to in this report:

D1: WO 97 30732 A (ONYX PHARMA INC) 28 August 1997 (1997-08-28)
D2: WO 00 29573 A (CANJI INC) 25 May 2000 (2000-05-25)

1. Amendments (Art. 34.2.b PCT)

The present report is based on the set of claims received on 22.04.04. Amendments have been accepted as supported and in accord with the description as originally filed.

2. Novelty (Art 33.2 PCT)

2.1 Subject matter of claims 1, 2, 4, 10, 11, 14-23 is not new. D2 discloses conditionally replicating adenoviral vectors, based on Ad5 (e.g. human), able to restore the $p53^+$ phenotype into target cells, by the presence of a p53 gene under a CMV promoter, and to induce this way apoptosis into cells (see page 32 line 17, to page 34, line 15, and examples 1 and 2). D2 also discloses its use in a method of treatment, coresponding to claims 14-23.

3. Inventivity (Art. 33.3 PCT) and essential features (Rule 6.3 PCT))

3.1 Regarding claim 3, the use of the selectivity of the promoter to target neoplastic cells, avoiding infection and replication in non neoplastic cells, is also disclosed in D1 (see abstract, see page 5, line 19, to page 6, line 10; see page 11, line 11 to line 29).

3.4 In view of the above points, subject matter claims, 7-9, 12 and 13, which are novel, also appear to be inventive. Essential features present independent claims and in the disclosure of the application (see page 14 of the application, for instance) are not derivable from the prior art.

4. Further considerations for a later national/regional phase.

4.1 Expressions like "preferably" in the claims are not limiting the subject matter. Their use does not limit the scope of the claim to said feature (eg. "serotype 5" in claim 2)

4.2 Claims 21-23, relate to methods of treatment, a subject matter which is excluded from patentability by the European Patent Convention (see EPC Art.52.4).

4.3 A document published after the claimed priority date, (Van Beusechem et al. Cancer Res. 62, Nov. 2002, 6165-6171), has been introduced during the international examination. Priority document EP 1327688 is similar to the present application, but lacks i.a. examples 6-12 and subject-matter of claims 9 and 13.

4.4 Claims 5 and 6 refer to a "fuctional analogue". A protein or a compound can have a plurality of functions. In this regard, the definition of "functional analogue" is not clearly defined.

Marchenko et al., J. Biol. Chem. 275(2000):16202-16212). There is evidence from mutation analysis that the transcription activation functions of p53 responsible for growth arrest and apoptosis can be dissected. For example, the p53 Q22/S23-mutant protein has abrogated growth arrest function but only attenuated apoptosis induction capacity (Venot et al., Oncogene 18(1999):2405-2410). On the other hand, several p53 amino acid 175 mutants were identified that retain cell cycle arrest function but are impaired in apoptosis induction (Ryan and Vousden, Mol. Cell. Biol. 18(1998):3692-3698). Furthermore, several p53 homologues have been identified, including p73 and p63, which share part of the functions with p53 (Kaghad et al., Cell 90(1997):809-819; Yang et al., Mol. Cell 2(1998):305-316). In the presence of the adenovirus E1B-19kDa protein, which binds to and inactivates pro-apoptotic death genes of the *bcl-2* family, the p53-dependent growth arrest pathway becomes apparent. Otherwise, apoptosis is dominant over growth arrest (Han et al., Genes Dev. 10(1996):461-477).

Recombinant adenoviruses, are finding increasing utility for the treatment of cancer and other diseases involving inappropriate cell survival. In particular, CRAds have been developed to selectively replicate in and kill cancer cells. Such cancer-specific CRAds represent a novel and very promising class of anticancer agents (reviewed by Heise and Kirn, *supra*, Alemany et al., *supra*; Gomez-Navarro and Curiel, *supra*). The tumor-selective replication of this type of CRAds is achieved through either of two alternative strategies. In the first strategy, the expression of an essential early adenovirus gene is controlled by a tumor-specific promoter (Rodriguez et al., Cancer Res. 57(1997):2559-2563; Hallenbeck et al., Hum. Gene Ther. 10(1999):1721-1733). The second strategy involves the introduction of mutations in viral genes to abrogate the interaction of the encoded proteins with cellular proteins, necessary to complete the viral life cycle in normal cells, but not in tumor cells (Bischoff et al., Science 274(1996):373-376; Fueyo et al., Oncogene 19(2000):2-12; Heise et al., Clin. Cancer Res. 6(2000):4908-4914; Shen et al., J. Virol. 75(2001:4297-4307). During their replication in tumor cells CRAds destroy these cells by inducing lysis, a process that is further referred to as "oncolysis". The release of viral progeny from lysed tumor cells offers the potential to amplify CRAds

Two94/18992; WO97/30732;

Application no. PCT/EP03/00340

in the name of Vereniging voor christelijk wetenschappelijk onderwijs et al.

NEW CLAIMS

1. Replication competent recombinant adenovirus, being capable to replicate and having lytic capacity in target cells, the said cells being hampered in the p53 dependent apoptosis pathway, the virus being a conditionally replicating adenovirus and comprising in the genome thereof, the coding sequence of at least one restoring factor functional in restoring the p53 apoptosis pathway in the said target cells, operably linked to one or more expression control sequences, functional in the said target cells.

2. Recombinant virus according to claim 1, wherein the virus is a human adenovirus, preferably of serotype 5.

3. Recombinant virus according to claim 1 or 2, wherein expression of at least one essential early adenovirus gene is controlled by a tumor-specific promoter.

4. Recombinant virus according to any of the preceding claims, wherein the adenovirus is a heterologously trans-complemented adenovirus.

5. Recombinant virus according to any of the preceding claims, wherein the virus genome comprises at least the gene encoding the adenovirus E1B-55kDa protein or a functional analogue or derivative thereof.

6. Recombinant virus according to claim 5, wherein the virus genome further comprises the gene encoding the adenovirus E1B-19kDa protein or a functional analogue or derivative thereof.

7. Recombinant virus according to claim 5 or 6, wherein the virus genome comprises one or more, preferably all, of the genes of the adenovirus E4 region encoding E4 proteins or functional analogues or derivatives thereof.

8. Recombinant virus according to claim 7, wherein the virus genome comprises at least the gene encoding the adenovirus E4orf6 protein or a functional analogue or derivative thereof.

9. Recombinant virus according to any of the preceding claims, wherein the adenovirus carries a mutation in the E1A region encompassing at least a part of the pRb-binding CR2 domain of E1A, preferably a deletion encompassing amino acids 122 to 129 (LTCHEAGF) of E1A.

10. Recombinant virus according to any of the preceding claims wherein the restoring factor is chosen from the group, consisting of p53, p63, p73, BAX, BAK, BOK/Mtd, BCL-X_S, Noxa/APR, PIDD, p53AIP1, PUMA,KILLER/DR5, Apaf-1, PIG, BID, tBID, BAD, HRK, Bik/Nbk, BLK, mda-7, p14ARF or a functional variant, analogue or derivative thereof.

11. Recombinant virus according to claim 10, wherein the restoring factor is p53 protein, preferably human p53, or a functional analogue or derivative thereof.

12. Recombinant virus according to claim 11, wherein the protein lacks a functional binding domain for the human MDM2 protein.

13. Recombinant virus according to claim 11 or 12, wherein the protein is a functional derivative of human p53 with mutated amino acids Leu-14 and Phe-19.

14. Recombinant virus according to any of the preceding claims, wherein the target cell is a human cell, preferably chosen from the group, consisting of cancer cells, arthritic cells, hyperproliferative vascular smooth muscle cells and cells infected with a virus other than the said recombinant virus.

15. Use of the recombinant virus according to any of the claims 1-14 in a medicament.

16. Use according to claim 15 for the manufacture of a medicament for suppressing uncontrolled cell growth, in particular malignant cell growth.

17. Method for lysing target cells hampered in the p53 dependent apoptosis pathway, comprising the steps of:

- infecting the said target cells with a virus, having lytic capacity in the said target cells,
- replicating the said virus within the said target cells, further comprising the step of providing, in the virus genome the coding sequence of at least one restoring factor, functional in restoring the p53 dependent apoptosis pathway, the said coding sequence being capable to be expressed in the target cells upon infection thereof by the said virus.

18. Method according to claim 17, wherein the target cells are infected by a recombinant virus according to any of the claims 1-14.

19. Method according to claim 17 or 18, further comprising the step of subjecting said target cells to irradiation and/or a toxic chemical compound.

20. Method according to any of the claims 17-19, wherein said target cells are present in an animal body, preferably a human body.

21. Method for treatment of a subject body suffering from a condition involving body cells hampered in the p53 dependent apoptosis pathway, comprising the step of administering to the said subject body an effective amount of the recombinant virus according to any of the claims 1-14.

22. Method according to claim 21, wherein the condition is associated with uncontrolled cell growth.

23. Method according to claim 22, wherein the condition is chosen from the group, consisting of cancer, arthritis, in particular rheumatoid arthritis, or vascular smooth muscle cell hyperplasia.